

COMMAND FIBRES IN THE CIRCUMOESOPHAGEAL CONNECTIVES OF CRAYFISH

I. TONIC FIBRES

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INTRODUCTION

Evidence is accumulating for the existence of interneurons in several invertebrates which are capable of driving specific behavioural outputs when electrically stimulated. The bulk of experimental information concerning these 'command fibres', as they have been termed, has been acquired from crustacean preparations. This expanding series began with the observation of Wiersma (1938) that the giant interneurons of crustaceans evoke the familiar escape responses. Subsequently, other systems were illustrated to be influenced by command-fibre stimulation: cardiac ganglion (Wiersma & Novitski, 1942), swimmerets (Wiersma & Ikeda, 1964), defence reflex (Wiersma, 1952; Atwood & Wiersma, 1967), tonic abdominal musculature (Evoy & Kennedy, 1967; Kennedy *et al.* 1967), and uropods (Larimer & Kennedy, 1969*b*).

Similar experiments have been performed in several other invertebrate preparations. In the locust apparently normal stridulation can be generated by stimulating electrodes implanted in the supraoesophageal ganglion (Huber, 1960) and flight by stimulation of nerve bundles isolated from the circumoesophageal connectives (Wilson, 1965). Specific large cells in certain gastropod molluscs, *Aplysia* and *Tritonia*, when stimulated electrically, can evoke swimming escape responses (Willows, 1968; Willows & Hoyle, 1968) and various gill movements (Kupferman & Kandel, 1969; Peretz, 1969).

In general, crustacean behaviour patterns evoked by command fibres have been found to be reproducible from individual to individual. Similarly, identified command interneurons have a relatively constant location within the cord in different individuals (Atwood & Wiersma, 1967; Wiersma & Ikeda, 1964; and others). The constancy of location and function suggests a genetic assignment of morphology and connectivity. In several instances the motor discharge pattern provided by a command fibre has been shown to be organized by the CNS elements in the absence of sensory feedback (Wiersma & Ikeda, 1964; Evoy & Kennedy, 1967; Wilson, 1964; Larimer & Kennedy, 1969*b*). However, when feedback is present it can modify the basic central pattern (Wilson, 1964; Davis, 1969). Command fibres themselves sometimes receive peripheral sensory input, but this is not universal. The general rule seems to be that those interneurons evoking the most complex outputs are least easily modified by general sensory input, suggesting that command fibres do not function simply as

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conventional multimodal sensory interneurons with synaptic connexions leading motor output. Another generalization that has been well documented is the presence of several related command fibres that evoke similar but not identical behaviour patterns, such as those that provide for various kinds of abdominal flexion, or extension (Evoy & Kennedy, 1967; Kennedy *et al.* 1967), uropod movements (Larimer & Kennedy, 1969*b*), swimmeret control (Wiersma & Ikeda, 1964; Davis & Kennedy, 1972*a, b, c*) and even escape (Wiersma, 1938; Larimer *et al.* 1971; Wine & Krasne, 1972). Indirect evidence has been presented (Davis & Kennedy, 1972*b*) for the simultaneous activation by the animal of several command fibres to account for range fractionation within a behaviour pattern, but in spite of extensive studies to date, particularly on crustaceans, it is not certain that 'command fibres' actually mediate the normal behaviour of these animals. Their role in behaviour remains largely undocumented because access to a known command fibre in an animal for purpose of recording is technically difficult. In addition, if simultaneous activity in several command fibres underlies most behaviour patterns, records from any single unit might be difficult to interpret. Regardless of how command fibres are ultimately found to be dispersed in the hierarchy of central neurons, they nevertheless offer a promising approach to studying both behaviour and central organization. An appropriate command fibre may offer a unique and reliable means of repeatedly activating an interesting behaviour pattern or of evoking movements that seldom appear in the repertory of a normally behaving animal (see Page & Sokolove, 1972).

Work to date on crustacean command fibres has been directed almost exclusively toward the abdominal nerve cord. This site has been preferred because it provides access both for isolation of interneurons and for recording motor output patterns. In addition, the nervous system at this level remains viable during lengthy experiments. Although the abdominal CNS includes cyclical outputs for the control of swimmerets and uropods (Wiersma & Ikeda, 1964; Larimer & Kennedy, 1969*b*; Davis & Kennedy, 1972), as well as centres for tonic positioning (Evoy & Kennedy, 1967; Kennedy *et al.* 1967), it does not provide the total range of outputs of which the CNS is capable. An understanding of the full complement of command fibres present in an animal requires that they be isolated from a site higher in the nervous system, e.g. the circumoesophageal connectives. Interneurons at this level course toward lower ganglia that incorporate the walking, swimming, and swimmeret oscillators, as well as the centres responsible for many positional outputs. It has already been shown by Atwood & Wiersma (1967) that some of the most interesting command interneurons yet described exist at this level. Most of their work, however, has been confined to low-frequency drives, to the effects of frequency changes on the outputs, and to the problems of establishing identities.

We have intentionally searched for interneurons in the circumoesophageal connectives that evoke widespread tonic and positional changes as well as for interneurons evoking phasic and cyclical behaviour patterns. This work required the use of higher frequencies of stimulation than had previously been employed in order to observe the full diversity of evoked behaviour. In an attempt at completeness, we have also made extensive use of cinephotography in order to analyse the outputs for identities of evoked response. This paper describes the various tonic positional drives obtained from circumoesophageal interneurons. We have, however, emphasized those behaviour

Patterns which involve wide segments of the animals' nervous system to the exclusion of the more limited drives. We have also attempted whenever possible to recognize the behaviour patterns that appear to be identical to those seen in earlier studies as a result of interneuron stimulation in the abdominal cord. A subsequent paper incorporates the prominent phasic and cyclical behaviour patterns that are evoked from circumoesophageal interneurons, with special attention to those evoking swimming and walking.

METHODS

(A) General features

The crayfish *Procambarus clarkii* (Girard), used in these experiments, were obtained locally, near Austin, Texas. The animals were suspended in approximately four litres of saline (van Harreveld, 1936) maintained at 12–15 °C; a high level of dissolved oxygen was also provided by continuous bubbling of oxygen through the bath. The experimental lifetime of a successful preparation under these conditions was 5–6 h, which was approximately the time required for a complete examination of bundles teased from one connective.

The animal was secured with epoxy glue by the left branchiostegite to the end of a rigid Plexiglass rod. During the initial experiments a platform which could be lowered or raised as desired, was provided as support for the appendages. Under these conditions, however, the generation of command-fibre-evoked walking was not apparent (see Bowerman & Larimer, 1973). More normal proprioceptive feedback was deemed to be important in coordinating and maintaining such walking drives (also see Atwood & Wiersma, 1967). Such a condition was achieved by providing a circular Plexiglass platform which could be rotated by the animal during normal forward or backward walking.

(B) Dissection and stimulation

A window was cut through the dorsal carapace from the cephalic groove to near the rostrum. Immediately subsequent to removal of the stomach and hepatopancreas the cavity was flushed repeatedly with oxygenated saline in an effort to expel potentially destructive digestive fluids. The medial and lateral cephalic arteries (Baumann, 1917; Maynard, 1960) were then ligated to minimize the low pressure shunt to the bath, thereby maintaining blood perfusion to the thoracic and abdominal ganglia. Both connectives were crushed near the brain to eliminate descending activity. The right circumoesophageal connective was de-sheathed and the lateral and medial giant fibres were removed. Small bundles of fibres were stripped from the connective and suspended on platinum hook stimulating electrodes. If on the basis of evoked output it was suspected that more than one command fibre was present, the bundle was subdivided and re-tested. Each nerve bundle was stimulated electrically with pulses of varying voltages (from 3–7 V) at a duration of 0.2 msec. In most instances stimulus frequencies of 50 and/or 75 per sec were utilized to evoke behaviour.

(C) Filming

When stimulation of a bundle repeatedly evoked a particular response at a clear voltage threshold, the behavioural output was filmed at 8 frames/sec with a Beaulieu

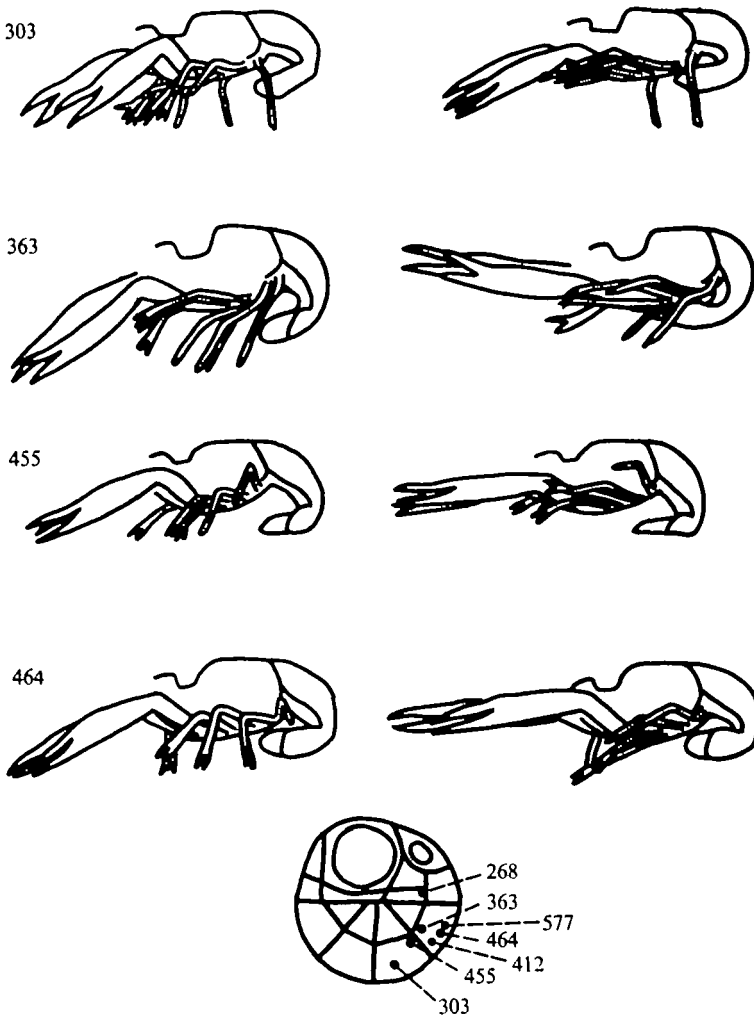


Fig. 1. General promotor command fibres. The crayfish profiles presented in this and subsequent figures have been traced from single frames, which were selected to illustrate the effect of tonic command-fibre stimulation. The identifying number of each general promotor command fibre is placed immediately to the left of an outline presenting the neutral, before-stimulation crayfish position. The drawing to the right is representative of the terminal evoked position. The stimulation frequency was 50/sec for C.F. 303 and 75/sec for each of the others.

On the cross-sectional map of the circumoesophageal connective, the locations of command elements within an identity are noted. Fibres for which profile information is not provided but which belong to the identity, are included in the map as well. It should be noted that two sets of data are combined for the ultimate establishment of a command-fibre identity: similarity of evoked posture for different command fibres and spatial proximity within the connectives.

16 mm camera on Kodak Tri-X film. Both lateral and overhead perspectives were utilized as needed. Film analysis was carried out with a stop-action projector (Kodak Analyst) which permitted profile tracing of single frames. A notation of command-fibre number and the stimulation frequency were recorded directly on to the cine film along with a stimulus-monitor light for accurate timing.

The approximate position of each command interneuron was marked on a cross-sectional map of the circumoesophageal connective (Wiersma, 1958). Groups of interneurons which are thought to be homologous i.e., established identities, are not tightly clustered on the cross-sectional map in certain cases (Fig. 1). Two separate factors may contribute to this scattering, but their relative effects are unknown. First of all, the assignment of axonal position was subject to experimental error. It took 3–5 h to sample a connective, during which time neural landmarks such as the giant fibres were lost and the orientation of the remaining aspects of the connective were obscured. Such factors contributed to a scatter of assigned positions even if fibres were teased from identical locations in different connectives. Secondly, there is no evidence that homologous units in different animals are not themselves scattered to a certain extent, being only approximately positioned in each organism. Both of these factors should be taken into account when viewing the command-fibre maps.

RESULTS

By the nature of the experimental procedure it was not possible to prove directly (e.g. by double isolation, Kennedy *et al.* 1966) that a specific behaviour pattern was the consequence of stimulating a single interneuron. Evidence for the existence of discrete command fibres was instead assembled indirectly. The major criterion for a single element was the presence of a sharp voltage threshold for evoking the complete behaviour pattern. When multiple fibres were responsible for a behavioural output, it was often possible to evoke the separate components by sequential recruitment of units as the voltage was progressively increased. Behaviour evoked by stimulation of several fibres was often not repeatable and could generally be described as poorly co-ordinated. Bundles containing multiple elements, however, could frequently be subdivided to provide several putative command fibres which apparently had interacted to produce the previously confused output. Additional verification of distinct command interneurons was obtained by pooling the data from many preparations since markedly similar behavioural outputs were encountered in different animals. It seems unlikely that such outputs are generated as a consequence of simultaneous stimulation of two or more specific interneurons since this would require that the same axons be stripped together in each preparation. If multiple command fibres were responsible for the behaviour patterns they would have to be in close apposition within a connective, each would have to survive the teasing process and have approximately the same threshold voltage. It should be noted, too, that in our experience fine bundles of only a few axons can be stripped more easily from the circumoesophageal connectives than from other levels of the CNS.

More than 500 command fibres were described from 28 animals in this and the subsequent study (Bowerman & Larimer, 1973). Each unit has been assigned a number together with information on its location in the connective and the behaviour pattern it evoked. The overall output was usually characterized on the basis of the positions attained by the chelipeds, walking legs, and abdomen in response to command-fibre stimulation. Tracings of positions before and after stimulation are given for several representative elements in each class, along with their location within the cord. Only when both the evoked behaviour pattern and the position within the cord

Table 1. *The table lists the command elements characterized in the present series of experiments, along with the corresponding animal from which they were obtained*

(The identifying command-fibre numbers appear in each of the subsequent figures. Note that certain animals yielded only 5-6 fibres while others were found to have more than 15. The rotating walking platform (see text) was used in preparations 15 to 28 inclusive.)

Animal	Command fibres	Animal	Command fibres
1	123-132	15	355-367
2	134-149	16	368-379
3	154-175	17	380-393
4	176-186	18	395-405
5	187-192	19	406-421
6	193-199	20	426-444
7	201-207	21	445-460
8	208-239	22	461-466
9	241-264	23	467-481
10	265-281	24	565-591
11	282-303	25	591-609
12	308-317	26	610-623
13	320-338	27	626-634
14	339-354	28	636-652

corresponded from several preparations was a collection of fibres considered an identity.

Information concerning command fibres located in each preparation is presented in Table 1. In many instances over 15 command interneurons were found in one individual, yet in other preparations, judged equally viable, less than ten were located. For the first 14 preparations the animals had a stable platform available for appendage support. In certain instances the platform was dropped, leaving the preparation freely suspended in the bath. The walking-wheel replaced the rigid support starting with preparation 15.

General promotor

A command interneuron which evoked general promotion of the limbs was discovered in at least seven different preparations, more than any other tonic fibre (Fig. 1). On the basis of the similarity of the behaviour patterns evoked in different preparations, and of the position of the axon within the circumoesophageal connective, these command fibres were considered to comprise an identity. When this interneuron was stimulated at 50 or 75/sec, the chelipeds were bilaterally lifted and extended directly in front of the animal with the chelae held partially open. The walking legs, especially the first three pairs, were bilaterally promoted and extended to a position approximately parallel to the long axis of the body. The final position attained by the last pair of walking legs was variable: restricted by the platform in 303, not lifted in 363; but lifted high in 455 and 464 (Fig. 1). The abdomen was flexed under the cephalothorax in all cases. In summary, stimulation of the general promotor command fibre streamlined the animal by bilaterally promoting the appendages and flexing the abdomen.

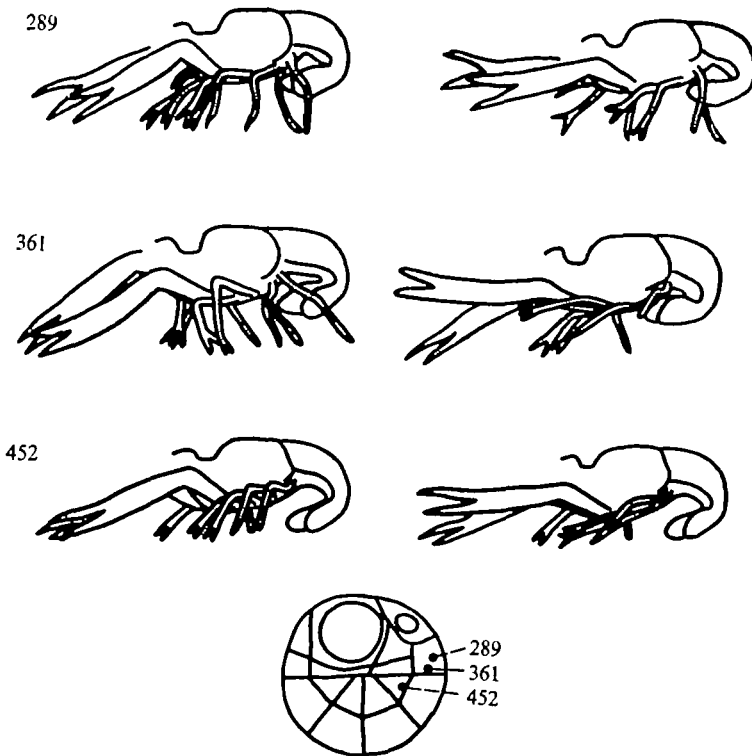


Fig. 2. Ipsilateral promotor. The three command elements presented in this figure evoke promotion of the ipsilateral appendages. A comparison of Figs. 1, 2 illustrates that the behavioural outputs and cross-sectional positions within the connective for these and the general promotor command elements are similar. The stimulation frequencies utilized to evoke the ipsilateral promotor postures were 75/sec for 289 and 452 and 50/sec for 361.

Ipsilateral promotor

The behavioural output generated by stimulation of each ipsilateral promotor command fibre (Fig. 2) was superficially similar to that generated by the general promotor. The abdomen was flexed under the cephalothorax and the ipsilateral appendages (with respect to the stimulated side of the cord) were promoted and extended to varying degrees. It should be noted that the ipsilateral effects were not as pronounced as with the general promotor and that the contralateral appendages were affected little if any by the command fibres. A strong indication that the ipsilateral promotor is distinct from the general was that both general and ipsilateral promotor units were found on several occasions in the same preparation. For example, fibres 303 and 289, 363 and 361, and 452 and 455 were found in animals 11, 15 and 21 respectively (Table 1).

Bilateral cheliped lift with abdominal flexion

The distributed positions evoked by stimulation of the three command fibres presented in Fig. 3 were similar in that chelipeds were lifted bilaterally and abdomens were flexed. The behaviour patterns differed from one another in the details of their

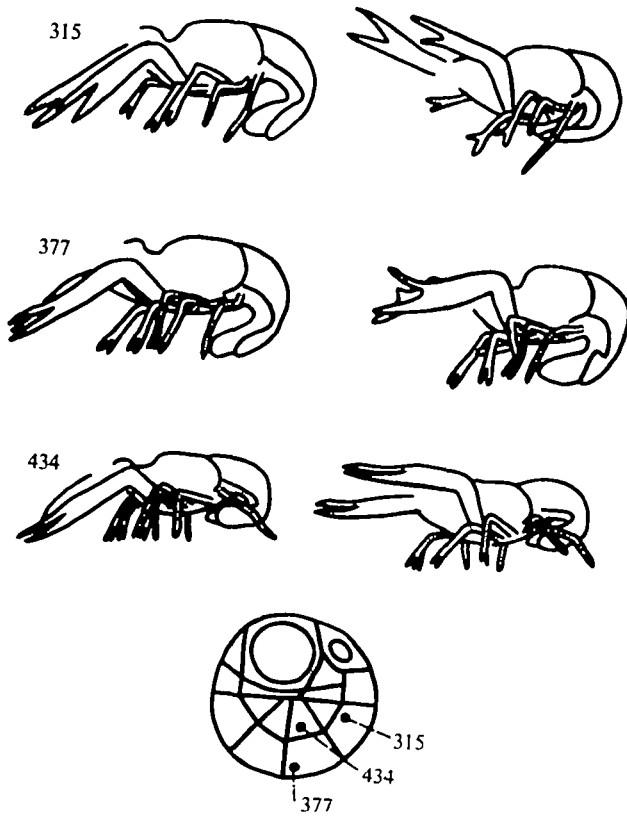


Fig. 3. The behavioural outputs evoked by stimulation of command neurons 315, 377 and 434 are similar in that the chelipeds are elevated and maintained and the abdomens are flexed. The different elements can be separated on the basis of command-fibre location within the connective as noted on the cross-sectional map and by the specifics of evoked output. Stimulation frequency was 75/sec in each instance.

final positions, as well as the location of the axons within the connective. For command drives 315 and 434 the cheliped positions were similar except that the chelae were opened in 315 and closed for 434. Stimulation of command element 377 not only evoked maximal opening of the chelae, but also positioned them in a medially-directed orientation. Additional positional variance was apparent in tail geometry; for fibre 315, abdominal flexion was fairly extreme and distributed over all abdominal levels, while fibre 434 evoked flexion that was restricted primarily to the most caudal segments of the abdomen. An intermediate position was attained when fibre 377 was stimulated, with the uropods being flared quite noticeably. The walking legs were little influenced by stimulation of these command elements.

Bilateral cheliped lift with abdominal extension

Body and limb geometry evoked by stimulation of command fibre 164 and two other elements from different preparations (not pictured) were unique (Fig. 4). Since the units were located close to one another within the cord, these were considered to be an identity. The chelipeds and first three pairs of walking legs were bilaterally lifted

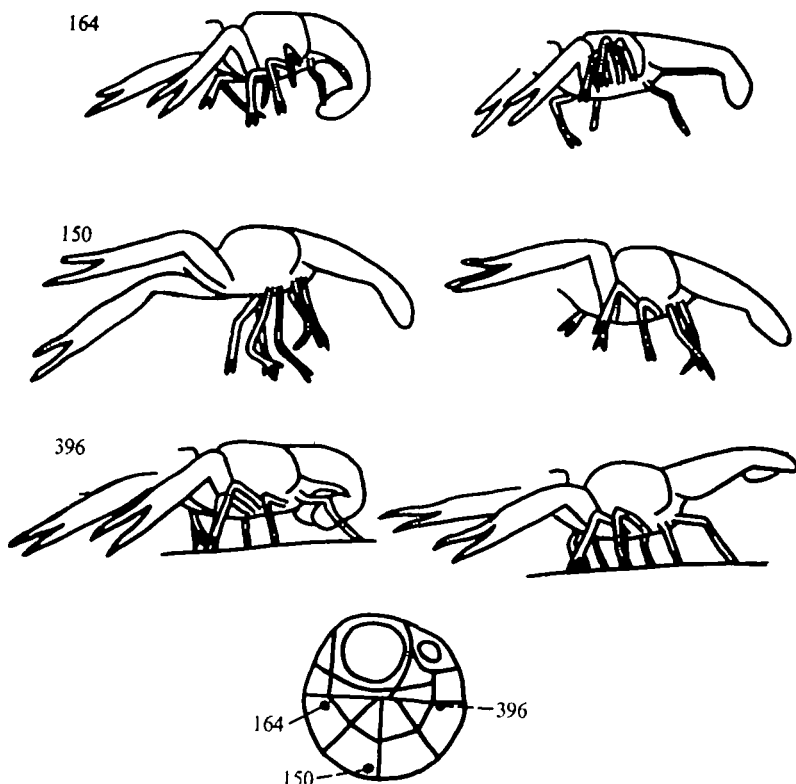


Fig. 4. Behaviour patterns evoked by command elements 150, 164, and 396 included bilateral lifting of the chelipeds in conjunction with abdominal extension. The outputs were differentiated on the basis of appendage position and location within the circumoesophageal connective. The frequency of stimulation was 75/sec in each instance.

and strongly flexed. Abdominal position was also characteristic, exhibiting rigid extension except for the telson. Interneuron 150 evoked bilateral lift of the chelipeds and the first two pairs of walking legs, while the terminal two pairs of walking legs were not obviously affected. Fibre 396 was of particular interest because it evoked a bilateral lifting of the chelipeds off the platform, and in addition extended the abdomen. This particular cheliped and abdominal position has also been noticed in conjunction with forward walking that was evoked by command fibres (Bowerman & Larimer, 1973).

For all command drives, cinephotographs provide data on the latencies between stimulus onset, initiation of movement, and attainment of final position. For the purposes of this paper, however, only a representative sample of this type of information will be presented. Fig. 5 illustrates positions attained sequentially at 1 sec intervals as a consequence of stimulation of command fibre 467 at 20/sec. It should be pointed out that, at this frequency, the fibre evoked only a defence posture, yet at 50/sec backward walking was also initiated. Latencies between stimulus onset and initiation of cheliped lift and between stimulus onset and abdominal extension were quite distinct from one another. Cheliped movement was well underway after 2 sec whereas abdominal movement was begun after about 2 sec of input. Movement durations to

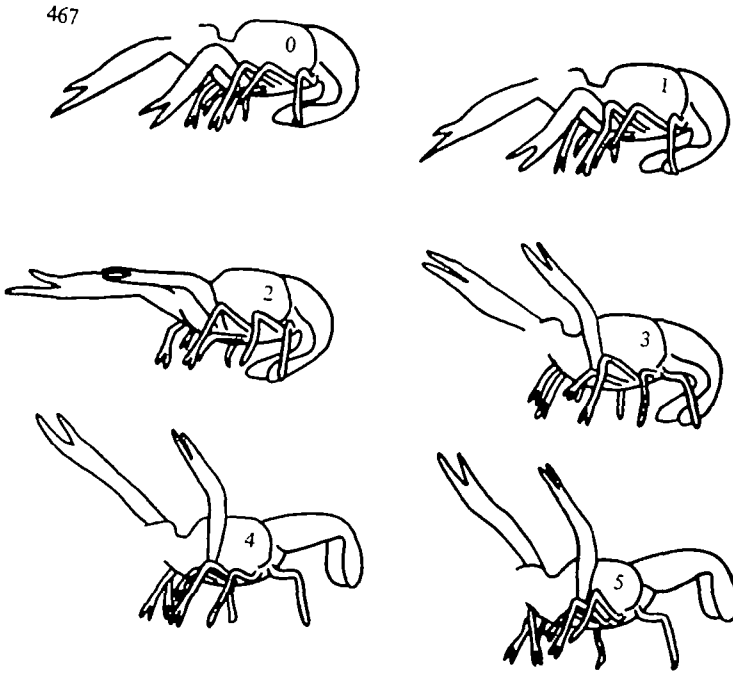


Fig. 5. Fig. 5 presents postural information for a 'defence' command fibre (467) which was only one of many such fibres. The stimulation frequency was 20/sec. Time (sec) subsequent to the onset of stimulation is given for each profile. A point of interest is the temporal fractionation of the behavioural program; i.e., separation of cheliped lift and abdominal extension performances.

terminal positions were also different; cheliped movement spanned 4 sec, and the terminal abdominal position was attained in less than 2 sec after it was initiated.

Ipsilateral lift and flex

These command fibres were grouped on the basis of their lift and flex effects on the ipsilateral cheliped and first three walking legs (Fig. 6). The drives were asymmetrical with contralateral appendages, particularly the walking legs, being minimally affected. The similarity of final commanded positions attained by the anterior four ipsilateral appendages in the different preparations was striking. In spite of the wide distribution of axon locations within the connective these fibres may well comprise an identity; this is uncertain, however.

General flexion

Command fibres placed within this category were capable of evoking a widely distributed body flexion (Fig. 7). That is, each command fibre caused bilateral flexion of all ten appendages together with varying degrees of elevation at the basal joints and abdominal flexion. It should be pointed out that for fibre 450 the platform restricted the attainment of the flexed position by the chelipeds and abdomen. Even though the final position of the walking legs differed in degree of elevation or rotation for th

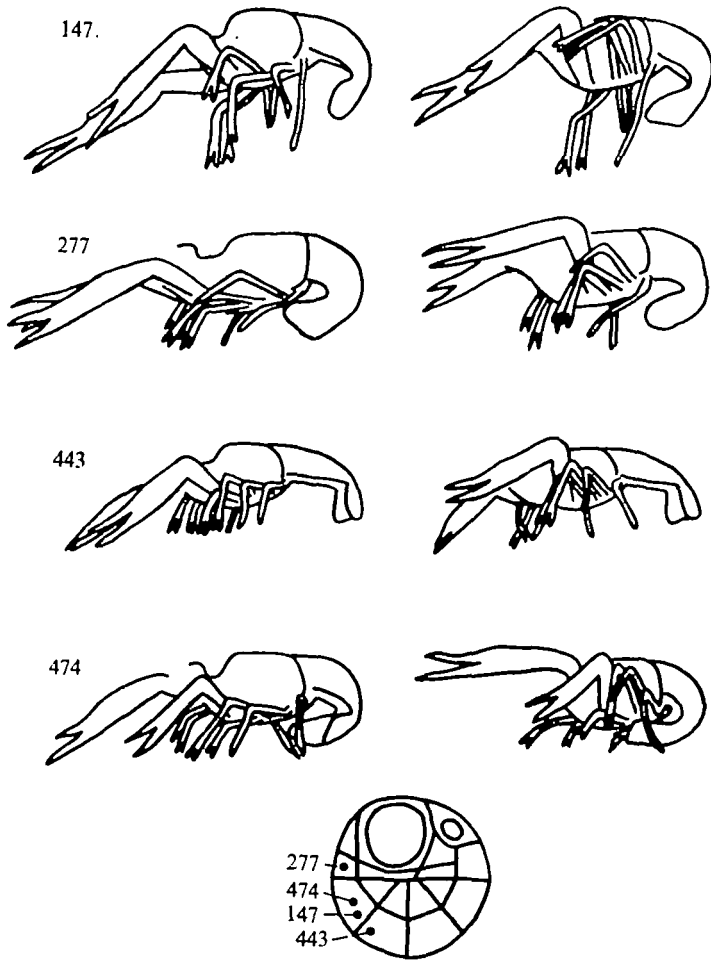


Fig. 6. The command fibres presented in Fig. 6 have been grouped on the basis of the lift and flex behaviour displayed by the ipsilateral appendages. A stimulation frequency of 50/sec was utilized for C.F. 147 and 75/sec for the remaining command elements. The relatively close spatial proximity of assigned command-fibre locations is noted on the cross-sectional map of the right circumoesophageal connective.

various command drives, it was felt that since the behavioural output was distinct (found only these four times), and the axons were in relatively close spatial proximity within the cord, these four fibres may constitute an identity.

Unilateral cheliped flexion

Command-fibre drives presented in Fig. 8 were similar only in the evoked activity of the ipsilateral chelipeds, differing from one another in the remainder of their output. Stimulation of fibre 314 generated an output similar to that of the lift and flex fibres (Fig. 6) but was located in a separate section of the cord. The ipsilateral cheliped and first three walking legs were lifted and the legs as well as the abdomen were flexed. The 4th ipsilateral and all contralateral walking legs were unaffected.

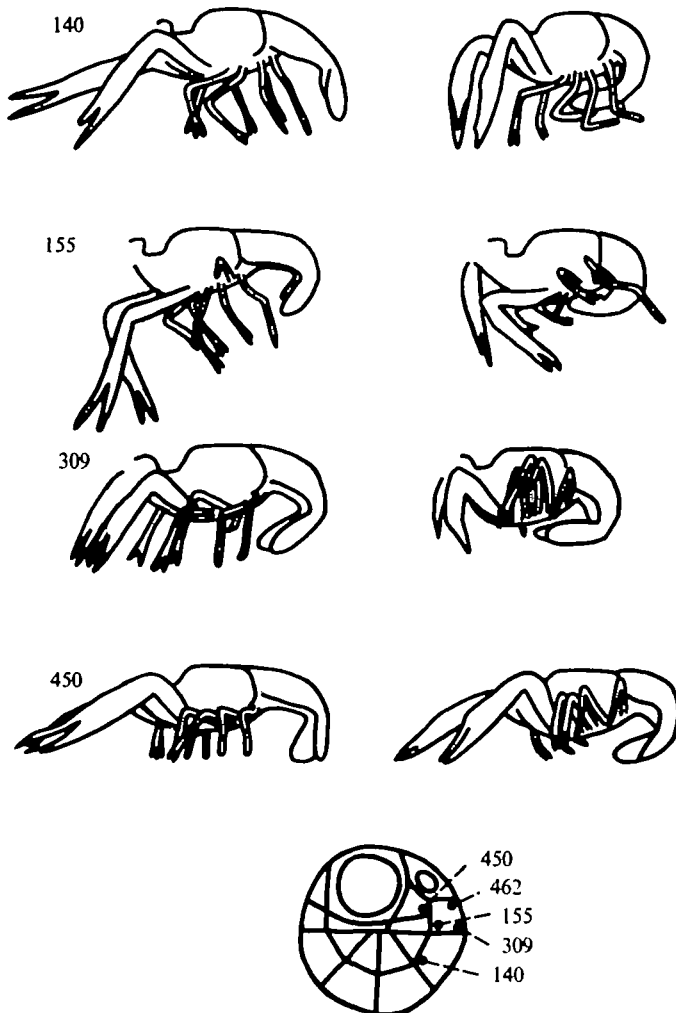


Fig. 7. Stimulation of command fibres presented in Fig. 7 results in a generalized flexion or a 'balling-up' of the preparation. A stimulation frequency of 50/sec was utilized for fibres 140 and 155 and 75/sec for 309 and 450. Even though a distinct variance exists between terminal appendage positions for the various command elements, it is felt they constitute an identity on the basis of their close spatial proximity within the connective. Also, no other general flexion command fibres were encountered at other locations.

Fibre 383 presented the interesting feature that the ipsilateral cheliped was flexed and the adjacent three walking legs were extended. It was uncommon for adjacent appendages to be influenced in such an opposite manner. Stimulation of 383 also resulted in the closing of the ipsilateral chela and the opening of the contralateral chela. In the case of 438, the ipsilateral appendages were highly flexed but not lifted, and both chelae were closed.

Cheliped-crossing

The first time this fibre was stimulated it was suspected that the evoked output was in fact the result of stimulation of two interneurons. Attempts to simplify the output, however, by subdividing the bundle or by careful searching for voltage

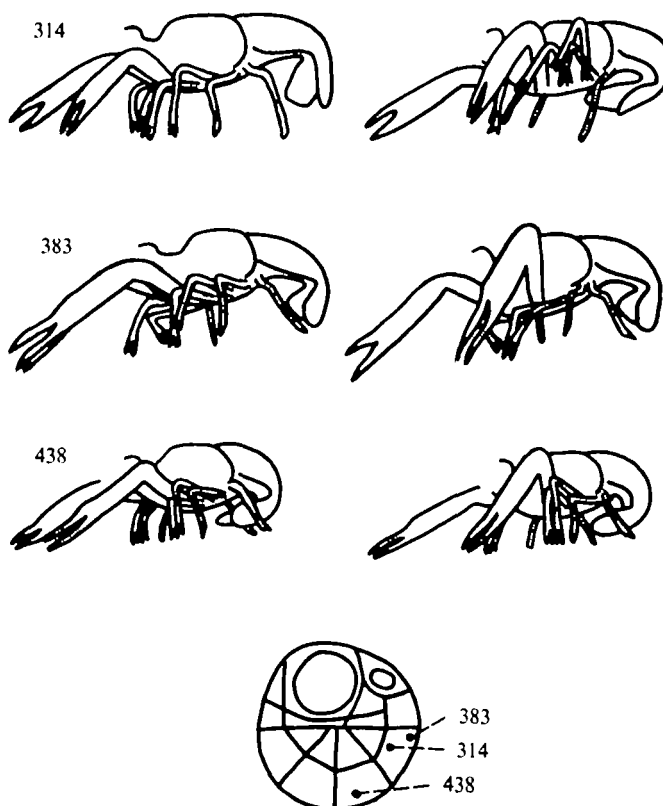


Fig. 8. Command fibres presented in Fig. 8 have been grouped on the basis of similarity of evoked cheliped movement, the ipsilateral cheliped being flexed in each instance. A stimulation frequency was 50/sec for C.F. 383 and 75/sec for 314 and 438. The positions of the command elements are noted on the cross-sectional map of the circumoesophageal connective.

thresholds failed to alter the effect. In subsequent experiments, the cheliped-crossing fibre was rediscovered on three occasions and the axons appeared to be in close proximity in the cord (Fig. 9). The terminal configurations generated by stimulation of the fibres were also nearly identical. The abdomen was set in a 'fishhook' geometry, i.e., extended rostrally and flexed caudally. Ipsilateral walking legs were lifted high and fanned out so that the first was oriented anteriorly and the last posteriorly. Contralateral walking legs were apparently unaffected. The most interesting component of the drive involved the positioning of the chelipeds. The cheliped ipsilateral to the experimental connective was first extended in front of the animal while the contralateral cheliped was lifted across it. All components of the behaviour pattern were present in each of the four cases, strongly suggesting that they were evoked by corresponding interneurons in different preparations.

Abdominal extension

The behavioural profiles in Fig. 10 illustrate the existence of interneurons in the circumoesophageal connectives which evoke rather marked abdominal extension. For each case the combined evidence of cross-sectional position within the connective

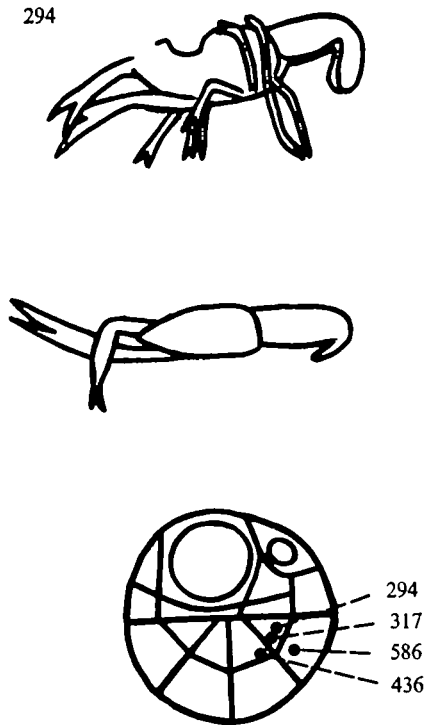


Fig. 9. The cheliped-crossing command elements have been found in four different preparations as shown on the circumoesophageal cross-sectional map. The evoked behaviour pattern was stereotyped with the abdomen being driven to a rostrally extended, caudally flexed geometry, the ipsilateral walking legs being lifted and fanned, and the two chelipeds crossing in a characteristic fashion. The profile drawings presented demonstrate these terminal positions as viewed from the side and from above. A stimulation frequency of 75/sec was used.

and total body position suggested strongly that these four units are distinct from one another. The caudal aspect of the abdomen, that is, the uropod and telson were maintained in a flexed attitude for drives 284 and 364. Stimulation of fibre 326, on the other hand, resulted in extension which also involved the uropods and telson. Only a few comments seem in order concerning the influence of each command drive upon the appendages. Stimulation of fibre 326 caused the appendages to exhibit non-specific movements, perhaps a function of lack of support. For 364, on the other hand, there was a tendency for bilateral lift and flex, particularly by the chelate appendages.

Turning fibres

Command interneurons assigned to this general category evoked one of two basic outputs, ipsilateral (286) or contralateral (318) turning (i.e., toward or away from the stimulated side) (Fig. 11). It may be of some interest that both turning modes are represented in a single connective. Many fibres existed which evoked the turning output with only two being presented as examples. Even though the ipsilateral and contralateral drives displayed a wide range of different specific characteristics, certain general features were apparent. For ipsilateral turns (fibre 286), ipsilateral cheliped and walking legs were remoted and the contralateral appendages promoted. In the

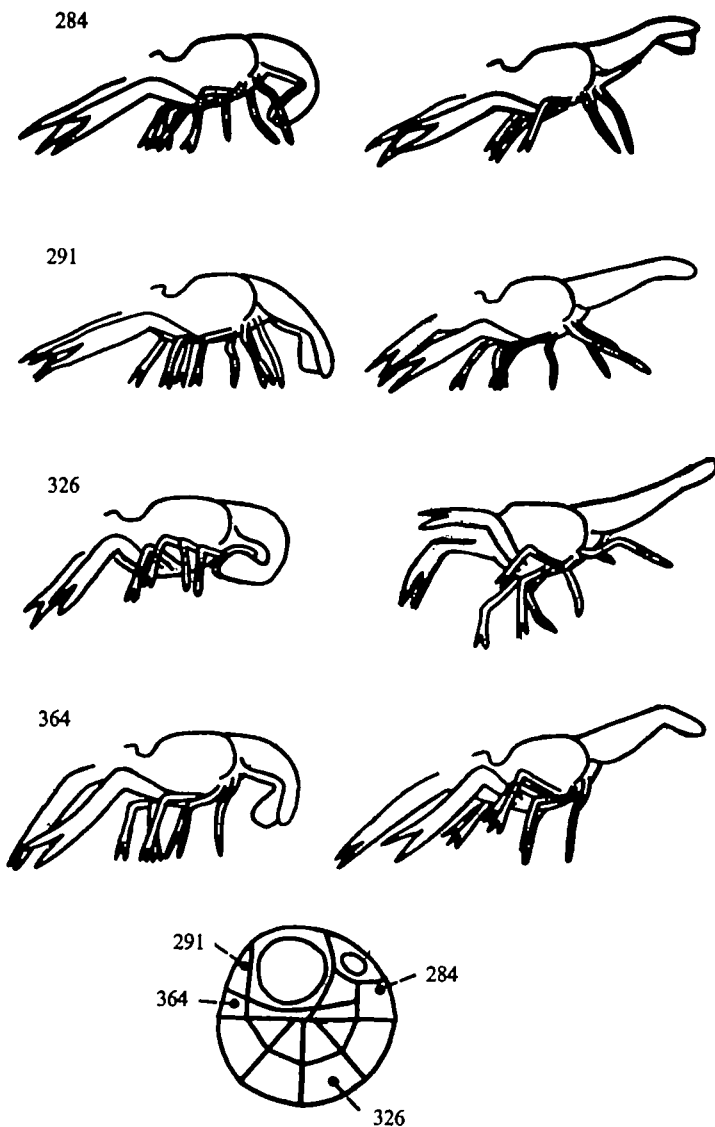


Fig. 10. Fig. 10 presents four command elements which evoked strong abdominal extension in conjunction with different appendage actions. Stimulation frequencies were 50/sec for 326, and 75/sec for the other command elements. The widespread dispersal of axonal positions within the connective clearly indicates that these fibres are not homologous.

majority of cases the remoting chela was closed and the promoting one opened. Accompanying this stereotyped positioning of the appendages were a variety of asymmetrical abdominal, swimmeret, and uropod actions. Even though the commanded output for a single fibre was constant, different elements within the larger class were quite individualistic. The abdomen, whether flexed or extended, was sometimes slightly rotated relative to the thorax. Asymmetrical beating of the swimmerets was also frequently seen to accompany turning behaviour. The swimmeret power stroke was directed laterally so that the appendages were clearly visible from a direct over-

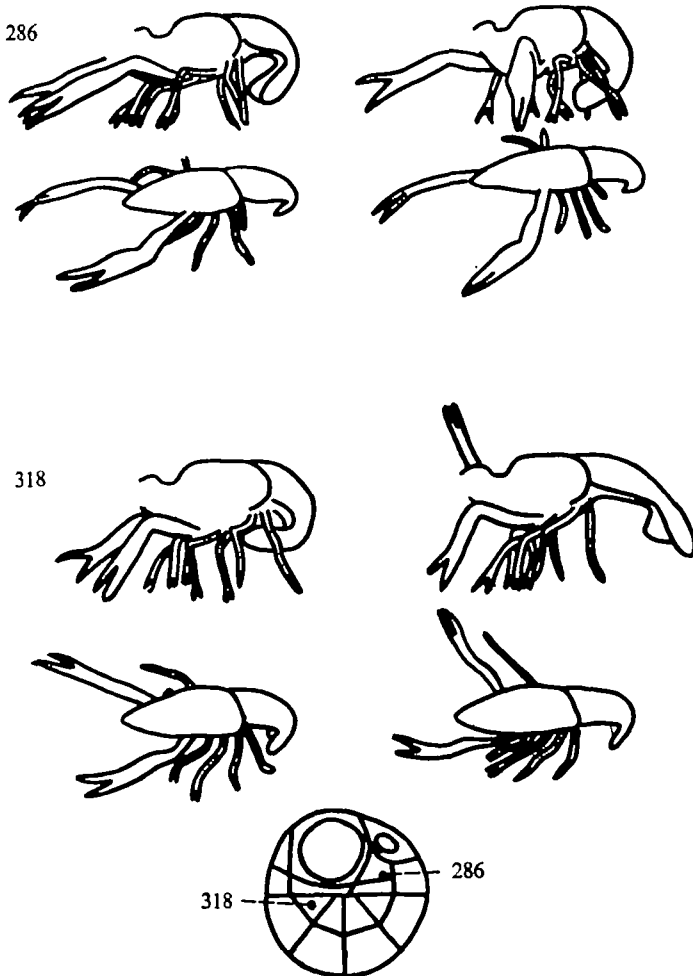


Fig. 11. Stimulation of fibre 286 at 75/sec resulted in ipsilateral turning; i.e. toward the side that furnished the command element. Simultaneous profiles from the side and from above are illustrated. Fibre 286 should be viewed as one of a class of fibres which when stimulated evokes the asymmetrical cheliped and walking leg movements characterized as turning.

Command fibre 318, when stimulated at 50/sec, generated contralateral turning. Again, this element is one of a group of command fibres which when stimulated evoke contralateral turning. Simultaneous profiles from the side and from above are provided.

head perspective. Turning behaviour often incorporated a flaring of one uropod and the remotion of the other. Complete descriptions of the various behaviour patterns obtained from stimulation of turning fibres awaits further experimentation utilizing synchronous cinephotography from above and from the side. Since the turning elements were not well documented, estimates of their total number are not available. One final point should be made concerning the term 'turning fibre'. This was adopted as a descriptive aid and should not be interpreted as an indication that these command fibres are actually involved in locomotory turning behaviour. Such may well be the case, but this has not been verified.

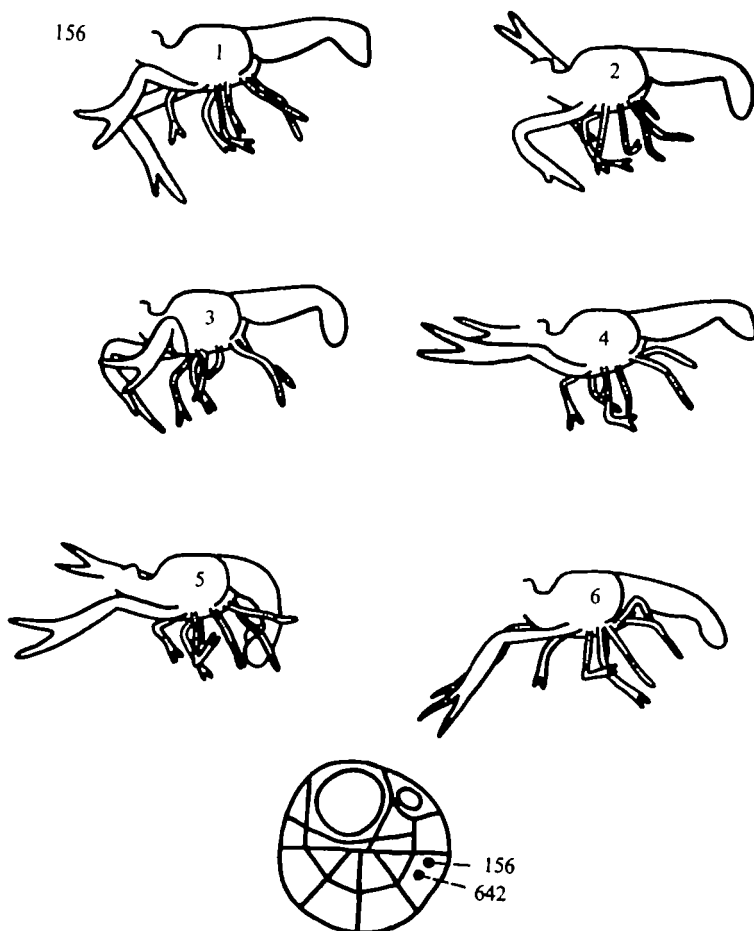


Fig. 12. Stimulation at 50/sec of command fibre 156 ('statue fibre') resulted in the cessation of ongoing movements. Fig. 12 presents profile drawings of six of these maintained positions from a preparation which was suspended freely in the experimental chamber and was exhibiting spontaneous activity. Upon termination of command-fibre drive the animal resumed activity and was then 're-frozen' in the new geometry. Positions presented in this figure were attained sequentially as numbered. The locations of command element 156 and of fibre 642 are noted on the cross-sectional map of the circumoesophageal connective.

The 'statue fibre'

A particularly interesting interneuron, which has been given the trivial name 'statue fibre', was observed only twice. The behavioural consequence of stimulating this particular command fibre was a suppression of all spontaneous movements, which, in effect, 'froze' the animal in position. An attempt at illustrating this feature of the output is presented in Fig. 12 (C.F. 156), where six different static positions (1-6) were sequentially commanded in a spontaneously active preparation. Spontaneous movement was suppressed within $\frac{1}{2}$ sec after stimulus onset; the resulting position was maintained for the duration of the test stimulus, i.e., for several seconds. A comparison of cheliped and abdominal positions in profiles one to six inclusive indicates that the movement-dampening influence of the interneuron was apparently not dependent

dent upon any particular limb of body position. Certain other features of statue-fibre activity were noted the second time it was observed. This preparation (animal 28, fibre 642) could easily be provoked into activity by mechanical stimulation. As was the case for 156, stimulation of fibre 642 also rendered the animal motionless. In addition, when fibre 642 was stimulated at 50/sec, tactile and proprioceptive inputs which had previously caused movement were no longer effective in provoking the animal into activity.

DISCUSSION

It has become evident from these and earlier experiments (Atwood & Wiersma, 1967) not only that a wide variety of different tonic command fibres exist at the circumoesophageal level, but also that the behavioural outputs of many of these elements are spacially distributed. It must be emphasized, however, that throughout the course of these experiments, command fibres which proved to evoke relatively complex, distributed outputs were specifically selected for filming and subsequent analysis. A number of equally specific command fibres affecting only the abdomen or a restricted number of appendages were noted but passed over in the current experiments. Such limited-output command elements isolated from the circumoesophageal connectives had been previously noted by Atwood & Wiersma (1967) and by C. Page (personal communication).

The bulk of earlier data on crustacean command fibres has been electrophysiological, gathered by stimulation of units in the abdominal nerve cords with records of neural output taken from various motor roots. The systems which have received special attention are those of abdominal position control (Evoy & Kennedy, 1967; Kennedy *et al.* 1967), the uropods (Larimer & Kennedy, 1969*a, b*) and the swimmerets (Wiersma & Ikeda, 1964; Davis & Kennedy, 1972*a, b, c*). Discussion relating the current work with previous experimental data is limited in certain respects since our data acquisition was exclusively cinematographic. Although extrapolation from neural to behavioural events may be equivocal, certain principles appear to be emerging.

From command-fibre experiments on the crayfish abdominal system arrays of flexion-evoking and extension-evoking interneurons were shown to be present (Evoy & Kennedy, 1967). Within one basic category, abdominal flexion, for example, evoked behaviour patterns could be differentiated on the basis of specific profiles of motoneuron recruitment within a segment as well as by the distributed output over several abdominal segments. A correspondingly extensive array of abdominal geometries can also be elicited by stimulation of elements isolated at the level of the circumoesophageal connectives (Fig. 13). In this experimental animal 18 abdominal geometries covering the complete range of tail settings were elicited: from complete extension (225), through intermediate flexion (211), to tight abdominal flexion (237). In several instances similar positions were achieved and maintained by stimulation of different command elements. Such data raise questions concerning the hierarchical organization of command elements. From information currently available it is not possible to differentiate between the case in which a command fibre exclusive for abdominal position is driven at the abdominal level by two or more convergent higher-order command elements and one in which parallel but completely separate neural circuits are responsible for similar abdominal geometries.

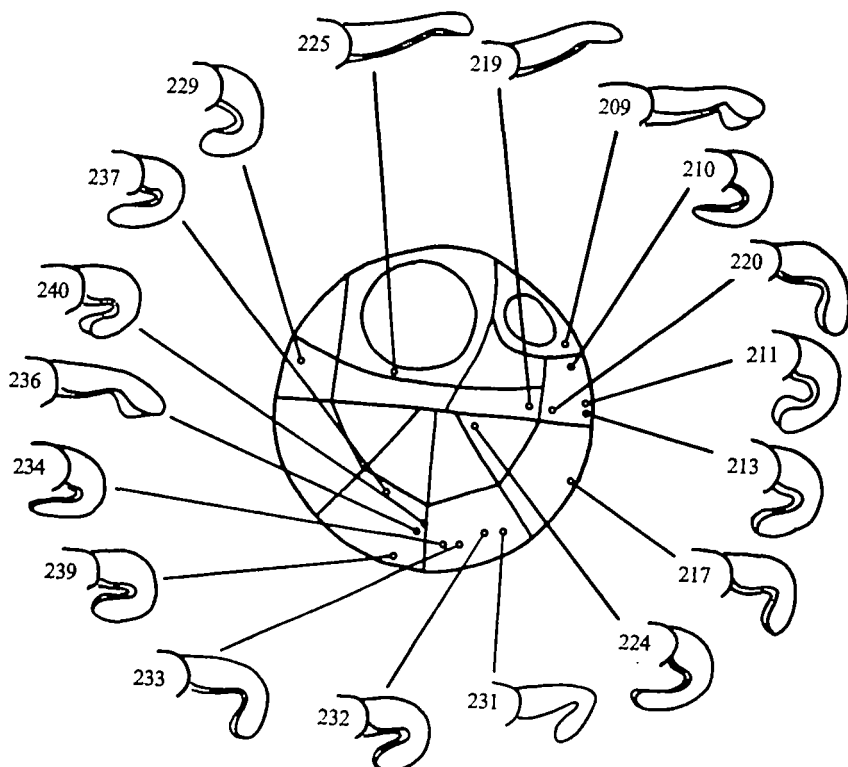


Fig. 13. This figure illustrates the number and range of abdominal geometries which were evoked by stimulation of tonic command fibres isolated from a single experimental animal, preparation number eight. Both the outline of the evoked abdominal geometry and the location of the responsible element within the connective are presented for each of the 18 tonic command fibres.

Studies have been made on the command-fibres systems controlling the swimmerets (Wiersma & Ikeda, 1969; Davis & Kennedy, 1972*a, b, c*) and the uropods (Larimer & Kennedy, 1969*b*). In the current work many command fibres were isolated which activated the swimmeret and/or the uropod control systems, but these outputs were largely neglected for several reasons. First of all, it was felt that the movements of the chelipeds, walking legs, and abdomen are more initially revealing in terms of behaviour contribution to the whole animal. Secondly, the mode of data acquisition (16 mm cine film at 8 FPS) was not well suited to assessing the finer elements of swimmeret and uropod control. Nevertheless, it was evident that the majority of previously described command fibres for the swimmerets, specifically excitation and inhibition, and for uropod positioning, both symmetrical and asymmetrical, were also accessible at the circumoesophageal level. An additional category of circumoesophageal command fibre was noted which evoked asymmetrical swimmeret beating characterized by a laterally directed powerstroke. Similar swimmeret activity can be observed upon occasion in intact crayfish when they are rotated about the longitudinal axis. In lobsters such redirected powerstrokes result in the generation of a righting torque (Davis, 1968). Accompanying the righting response were asymmetrical deployments of the uropods, and often, but not invariably, a rotation of the

abdomen relative to the thorax (Davis, 1968). The 'cheliped crossing' behaviour pattern, accompanying asymmetrical movements of the walking legs, and the abdominal geometry seen in our evoked drives (Fig. 9) also bear a striking similarity to the appendage movements that accompany righting behaviour pattern in the lobster (Davis, 1968). It is apparent that the various command influences which have been assessed in the crayfish abdomen can be isolated from the circumoesophageal connectives and are most probably utilized in conjunction with command influences involving the more anterior thoracic appendages.

It seems quite possible that tonic command fibres are functionally operative in at least one of several capacities. Certain elements, such as the general promotors, the defence posture fibre (467), and the cheliped-elevator fibres (Fig. 3), could well be used to generate and maintain discrete postures. This is an appealing hypothesis since evoked tonic output may resemble to a considerable degree a spontaneously generated crayfish posture. The great majority of tonic command fibres, however, evoke postures which are not reminiscent of known behaviour patterns. One may speculate that these elements, exemplified by turning fibres, ipsilateral promotors, and ipsilateral appendage lift and flex fibres, are normally operative as just one unit in an ensemble of active command fibres. Following this line of thinking it is proposed that maintenance of the majority of postural attitudes is the consequence of simultaneous activation of a discrete and specific ensemble of tonic command interneurons. On the other hand, tonic command-fibre discharge may superimpose a discrete bias on the neural read-out of a phasic command element, thereby acting as a behavioural modulator. Simultaneous activation of a forward locomotion fibre (see Bowerman & Larimer, 1973) and a turning fibre would effect an actual turn. Similarly, the general promotor command fibre may be operative during escape by ensuring an appropriate streamlining effect for backward locomotion. Additional possibilities for the possible employment of individual command interneurons could be offered, but in view of the relative lack of information on normal behaviour and on the role of command fibres in behaviour in general, it would appear that further speculation is unwarranted.

Statue fibre

The existence of a generalized movement-suppressing command fibre, ultimately termed the statue fibre, proved quite intriguing. One might postulate that this particular command unit, perhaps in conjunction with its contralateral homologue, is functionally operative in the capacity of a behavioural switch. In situations where either the rapid damping of ongoing activity or the ability to ignore provoking mechanical, visual, or tactile stimuli are critical, the appropriate behavioural over-ride could be insured by the specific activation of the statue fibres. Such behaviour, known as death-feigning or thanatosis, has been described in several arthropods particularly insects (Godden, 1972). Another conceivable role for this type of command element may be that of maintaining a specific postural configuration for long periods. Once a body geometry has been established by a central programme, maintenance of the posture could be turned over to statue-fibre elements.

Responses evoked by the statue fibre are somewhat reminiscent of the effects of the suppression fibres described by Evoy & Kennedy (1967). It is of interest that

Suppression fibres were often seen to break the strict reciprocity between flexor and extensor drives that are otherwise typical of the abdominal system. This type of connectivity may be in part a clue to the mechanism of action of the statue element. If the interneuron were capable of firing antagonistic motor units throughout the animal it could be responsible for the locking of joints at particular positions. This does not explain the positional independence, however. Alternatively, the fibre may turn on a wide array of resistance reflexes thus maintaining limb positions against gravitational forces. An understanding of the mechanisms of statue-fibre control awaits experimentation designed specifically toward an assessment of motor activity during evoked behaviour. It seems clear, however, that the effects are not achieved by simple but widespread inhibition of motor output, since this should result in a limp configuration dominated by the influence of gravity.

SUMMARY

1. Several discrete postures have been generated by electrical stimulation at frequencies of 20–75 Hz of command interneurons within the circumoesophageal connectives of the crayfish. The behaviour patterns were recorded by cine photography. The similarity of the patterns in different individuals generated by stimulation of interneurons found in the same positions within the cord, strongly suggested that certain of the interneurons examined in this study were identical.

2. A number of tonic command fibres were found, each of which evoked a specific body geometry. Tonic command units were classified on the basis of evoked cheliped, walking leg, and abdominal positions. The study concentrated on those command fibres which generated spatially distributed behaviour patterns involving both abdomen and appendages, and neglected command elements with more restricted outputs.

3. The types of tonic command fibres characterized include the following: bilateral appendage promotion, ipsilateral appendage promotion, bilateral cheliped lift, ipsilateral appendage lift and flex, general flexion, abdominal extension, and both ipsilateral and contralateral turning.

4. A movement-suppressing command fibre, ultimately termed the 'statue fibre' was found twice. Stimulation of this element terminated spontaneous activity by 'freezing' the animal, irrespective of position. An additional consequence of stimulation of the statue fibre was the overriding of the excitatory effect of mechanical stimulation which in the absence of the command drive would provoke the animal into activity.

5. It is suggested that a restricted number of tonic command fibres could be functionally operative in the generation of their corresponding behaviour patterns. For the majority of tonic command fibres, however, it is felt that the unit is merely one of a group of elements each of which is activated simultaneously or sequentially for discrete behavioural performances.

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